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The Potential of Algae *Spirogyra peipingensis* to Produce Nanogold in Batch Culture Medium

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Abstract

The biosynthesis of gold nanoparticles by using algae *Spirogyra peipingensis* was conducted. This research aimed to determine biosynthesis of gold nanoparticles by using algae *Spirogyra peipingensis* with different concentration and incubation time. The synthesis of gold nanoparticles using HAuCl_4 solution with variations of concentration 5 ppm, 10 ppm, 15 ppm and 20 ppm respectively in 1 liter of aquabidest. Algae *Spirogyra peipingensis* was grown in HAuCl_4 medium with the addition of 0.2 gr Sulfahri-01 nutrient. Then each 5 gram *Spirogyra* algae incubated in HAuCl_4 medium with the addition of Sulfahri-01 0.2 gram nutrients. 4 hours long incubation under exposure to sunlight. Nanoparticle size determination is done by looking at the color that appears in the solution. From the results of the research, it is known that *Spirogyra peipingensis* algae is able to synthesize gold nanoparticles characterized by the color change in algae biomass from green to purple color after being treated and forming gold nanoparticles with size 40-60 NM. The best-used HAuCl_4 consent is 5 ppm with the smallest particle size.

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Keyword

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Introduction

The development of nanotechnology applications has driven the industrial revolution globally. It has been reported that more than 400 companies worldwide have undertaken nanotechnology research and development activities. As a relatively new discipline, globally nanotechnology research and applications are growing very rapidly (Hoerudin & Irawan, 2015). Nanoparticles are a scope of nanotechnology. It is called a nanoparticle when the particle size is in the range of 1-100 NM. With unique properties and sizes, nanoparticles have great potential as a material that is superior in the future. The nanoparticle material can be organic material such as 2.5 NM DNA, 50 NM protein, 100 NM Virus type (Pantidos & Horsfall, 2014), or inorganic materials such as semi-conductor

nanoparticles (titanium oxide and zinc oxide), and precious metal nanoparticles (silver and gold) (Shaileyee & C Mandal, 2014).

According to Rohiman *et al* (2014), in the world of nanotechnology, research on gold nanoparticles has attracted the attention of many world researchers because of its superiority in its various applications. Gold nanoparticles (AuNPs) are gold ions that are reduced to non-charged gold and nano-sized (Maruani, 2013). AuNPs have prominent advantages compared to gold particles, which have optical and electronic properties with low toxicity (Rohiman, *et al*, 2014). In addition, AuNPs can be used as a water purifier (Qian, *et al*, 2013), carriers of cancer-killing drugs (Alhalili, *et al*, 2017), chemical and biological sensors (Qin, *et al*, 2018), catalysts (Amdouni, *et al*, 2018), biohydrogen production and various biomedical applications (Zhang & Shen, 2007). The production of AuNPs can be carried out by various chemical methods, but the production of eco-friendly nanoparticles are being intensively developed (Sovawi, *et al.*, 2016) through biosynthetic pathways using biological agents such as yeast, fungi, bacteria, plants, plant extracts and algae (Thakkar, 2010).

Algae are thallophyta organisms that have been known to have a lot of potential. One of the most easily found algae in Indonesia is *Spirogyra peipingensis* algae. This algae has the potential to reduce both organic and inorganic compounds. Based on the Bahri (2017) study, *Spirogyra peipingensis* algae can significantly reduce toxicity, absorbing heavy metals (Gupta & Rastogi, 2008), and reducing textile waste (Özer, *et al.*, 2006) that have mechanisms such as activated carbon. The use of algae as a gold metal bioreductor has previously been done by Roychordhury (2016). Algae used derived from the group of prokaryotic algae (*Cyanobacteria*) namely *Anabaena sphaerica* and eukaryotic green algae namely *Chlorococcum infusionum*. The alignment of cell components in algae such as carotenoids, polysaccharides, proteins and pigments in chloroplasts / thylakoids has a major role as reducing agents in the biosynthesis of nanoparticle metals (Roychordhury, *et al.*, 2016). carotenoids, polysaccharides, proteins and chloroplast pigments are also found in *Spirogyra* algae (Tipnee, 2015). Therefore, *Spirogyra peipingensis* algae is believed to have the potential to reduce gold particles to nano-sized gold particles.

Materials and Methods

Algae Preparation

Alga *Spirogyra peipingensis* obtained from the rice field area around the city of Makassar. then the algae is cleaned and identifiable under a light microscope before being cultured on growth medium sulfahri-01 (3000 lux, 12:12) which later becomes algae stock during the research phase.

Preparation of H₂AuCl₄ Solution

0.1 grams of gold metal dissolved into 20 ml aquaregia (3HCl: 1HNO₃). heat over hot plate until completely dissolved. then dilute it into a 500 ml measuring flask to make a parent solution of 200 ppm. The H₂AuCl₄ gold mains solution was diluted with aquabides with variations in dilution concentration of 5 ppm / L, 10 ppm / L, 15 ppm / L, 20 ppm / L in 1000 ml aquarium.

Incubation of algae *Spirogyra peipingensis* into gold medium

5 gr of *Spirogyra peipingensis* biomass were incubated on HAuCl₄ gold medium and 0.2 gr of additional sulfahri-01 nutrient media under exposure to 4 hours of sunlight. The top of the aquarium is covered with a cling wrap that is sufficiently ventilated for air circulation.

Determines the size of gold nanoparticles

After *Spirogyra peipingensis* algae incubation for 4 hours, the yellowish amorphous media solution was changed. The color variations formed can be used to determine the particle size of the synthesized product. This refers to the color guide of the solution to determine the size of the gold nanoparticles published by Noutarianni (2013).

Results and discussion

Algae Preparation

Fresh algae biomass *Spirogyra peipingensis* taken in the rice fields Sudiang, Makassar, Indonesia. then cleaned and identified under a magnification light microscope 100 times above Sedgwick Rafter Counting Chamber (SRC). Determination of *Spirogyra peipingensis* algae type was based on the publication (Zarina, et al, 2007) with unbranched filamentous morphology, with vegetative cell width 104-157 µm and length 156-200 µm and 5-7 pyrenoid. After identification, then Alga *Spirogyra peipingensis* in culture in 0.2 gr / L medium sulfahri-01 with water height 20 cm at 3000 lux lighting lamp with a duration of 12:12. The composition of the media has been tested based on Sulfahri, et al, (2016). From several variations of water height (10,20,30 cm), 20 cm is the most ideal water level with the highest level of productivity.

Algae Incubation

5 gr Biomass Algae *Spirogyra peipingensis* was incubated in a medium composed of 0.2 gr/L sulfahri-01 in several concentrations of HAuCl₄ with a series of 5 ppm, 10 ppm, 15 ppm and 20 ppm. Each solution was placed in a 20 cm glass tank with a capacity of 1000 ml and placed under 4 hours of sun exposure. The sulfahri-01 medium is the most superior medium among several types of mediums compared to having high nitrogen content which may play an important role (Sulfahri, et al, 2016)

After incubation for 4 hours, a change of color occurs in *Spirogyra peipingensis* biomass. The algae biomass, which was initially light green, became dark purple and was confirmed to have died from exposure to the metal mixture in its incubation medium (Fig.2a,2b). This change indicates that algae *Spirogyra peipingensis* can positively synthesize. This is in accordance with the results obtained by parial, et al (2012) who trace the types of *Phormidium valderianum* algae, *P. tenue*, *Microcoleus chthonoplastes*, *Rhizoclonium fontinale* and *Ulva intestinalis*. These five algae can positively synthesize intracellular gold nanoparticles. According to parial, et al, (2012) the five algae potentially as "Bionanofactories" and this is evident from the visual transformation of the algal thalli from green to deep purple.

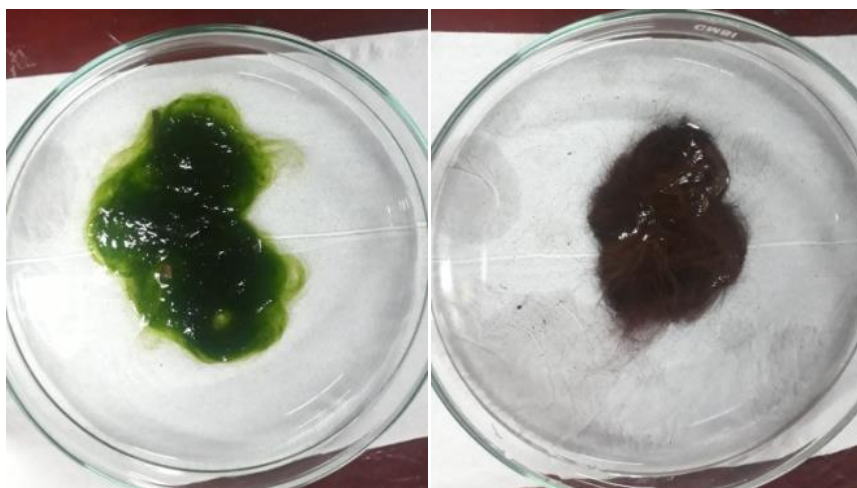


Fig. 2a. Before Incubation

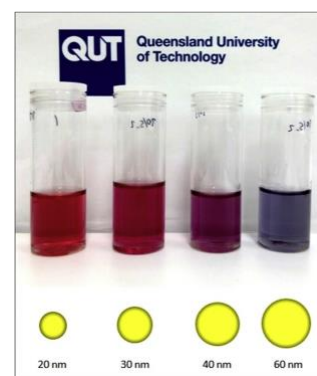
Fig. 2b. After Incubation

The ability of algae as a reducing agent to synthesize gold nanoparticles is much in play by the variety of cellular components such as carotenoids, polysaccharides, proteins and complete pigments in chloroplasts / tilakoid (Royconduri, et al, 2016). The reduction of the metal ions occurs upon the surface of the algal cell as well as on the cytoplasmic membrane, leading to the formation of gold nanoparticles (Senapati, et al, 2012). The exact process of intracellular formation of gold nanoparticles by alga biomass is not yet fully understood (Parial, et al, 2012).

Determines the size of gold nanoparticles



Fig. 3a. variations colour of media solutions



3b. GNPs sizing guide

In this way (Fig. 3a), it is possible to deduce the size of the Au NPs produced, ranging from 40-60 nm. This refers to the GNPs sizing guide based on the color of the solution (Fig.3b) from Queensland University of Technology (Notarriani, 2014). From this research, it can be seen that the bigger HAuCl_4 concentration, then the size of GNPs in the file will be greater because the particle size generated in the concentration series 5,10,15,20 ppm respectively contribute the change of color dry awaiting young to blue, which describes the existence the addition of the diameter of the nanoparticles, therefore, the 5 ppm concentration is considered the best as an incubation medium in producing gold nanoparticles.

According to Parial (2012), All applications of gold (or other) nanoparticles thus demand a well-defined size and accurate measurement of these parameters is correspondingly important. The optical property of surface plasmon resonance is directly

dependent on the size of the gold nanoparticle: ~20nm particles show an orange-red colour but the colour gradually shifts to blue when particle size increases to ~100 nm. Plasmon resonance also changes with aspect ratio.

Conclusions

From the study, it can be concluded that *Spirogyra peipingensis* algae can synthesize gold nanoparticles. Incubation of HAuCl_4 medium 5 ppm 0.2 g/L Sulfahri-01 best produces gold nanoparticles. From the color of the solution obtained, can also be concluded the smaller the concentration of HAuCl_4 the smaller the size of the particles produced. The intracellular gold nanoparticles biosynthesis has great potential to develop to industrial scale because it is easier, cheaper, and environmentally friendly.

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